

red blood cells contained in said sample undergo hemolysis during the above pretreatment and hemoglobin is released, and the denatured hemoglobin may interfere with measurement by binding to the virus antigen such as the HCV core. Thus, in the first embodiment of the present invention, it is preferred to remove the interference with measurement by capturing the heme in the hemoglobin. As an additive for this purpose, we have found that the addition of at least one of urea and a compound containing an imidazole ring is preferred.

As the imidazole ring-containing compounds, there may be mentioned imidazole, histidine, imidazoleacrylic acid, imidazolecarboxyaldehyde, imidazolecarboxamide, imidazoledione, imidazoleedithiocarboxylic acid, imidazoledicarboxylic acid, imidazolemethanol, imidazolidinethione, imidazolidone, histamine, imidazopyridine, and the like.

As the indole ring-containing compounds, there may be mentioned tryptophan, indoleacrylic acid, indole, indoleacetic acid, indoleacetic hydrazide, indoleacetic methyl ester, indolebutyric acid, indoleacetonitrile, indolecarbinol, indolecarboxaldehyde, indolecarboxylic acid, indoleethanol, indolelactic acid, indolemethanol, indolepropionic acid, indolepyruvic acid, indolyl methyl ketone, indolmycin, indoleacetone, indomethacin, indoprofen, indoramine, and the like.

The amount added is preferably 0.5M to 5M for urea, 5mM to 50mM for indoleacrylic acid, and 0.05M to 0.5M for the other additives.

On the other hand, membrane proteins such as the HCV coat protein do not dissolve spontaneously unless they are treated to that end. In order to dissolve a protein having a hydrophobic portion in water, the method of converting a hydrophobic portion into a hydrophilic portion with a surfactant is well known. It is known, however, that certain salts such as guanidine chloride have a property of making refractory proteins water-

soluble. Ions produced from salts (chaotropic agents) having such a property are called chaotropic ions, and as the anionic ions, guanidine ions, thiocyanate ions, iodine ions, periodate ions, perchlorate ions, and the like are known. Salts that generate these ions have been used for solubilization of refractory proteins. It was estimated that chaotropic ions have a function of efficiently releasing the antigens from the virus particles.

When a chaotropic ion is added, however, the secondary structure of proteins is disrupted causing the destruction of the epitope structure. Thus, when a probe such as antibody is added for the reaction of immune complex formation in the presence of a chaotropic ion as it is, binding with the antibody is weakened and the sensitivity decreases, which are thought to pose a serious problem.

On the other hand, the denaturing effect of chaotropic ions is mostly reversible, so that by weakening ionic strength by dialysis or dilution the denatured structure temporarily returns to the original structure. This poses another problem associated with the use of a treatment agent such as a chaotropic ion. That is, according to the desired treating method of the present invention, not only the virus particles present in the sample are efficiently released, but the high-affinity antibody that binds to the antigen present in the sample must be inactivated at the same time. Thus, solubilization with a chaotropic ion does not provide an adequate inactivation of the high-affinity antibody present in the sample, and, it is believed, the antibody adversely affects sensitivity.

Thus, the treating methods that employ chaotropic ions have two conflicting problems: in the condition in which a chaotropic ion can destroy a structure, the antigen-antibody reactions are inhibited, and on the other hand the effect of a chaotropic ion alone is not

sufficient to inactivate antibodies that interfere with reactions in the sample, and in the condition in which the antigen-antibody reactions are not inhibited, contaminating antibodies can inhibit the reactions.

5        In order to solve these conflicting problems it is necessary to find a condition in which the epitopes of the antigen are destroyed reversibly and the functions of the contaminating antibodies in the sample are destroyed irreversibly.

10      As to the conditions in which antibody is inactivated, an alkali treatment, an acid treatment and the like are known. The acid treatment of serum can cause false-positive results since the treatment irreversibly denatures some of serum proteins resulting  
15      in the formation of precipitates that in most cases hinder pipetting after the treatment of samples, and precipitates that engulfed the denatured proteins are adsorbed to the solid phase at the time of measurement and thereby may be detected as a density. In addition,  
20      another problem arises because when the antigen of interest is nonspecifically engulfed in the precipitate, the amount of antigen that reacts with the probe decreases resulting in a decrease in sensitivity.

25      The inventors of the present invention have found that the acid treatment combined with the guanidine treatment can resolve the problems associated with the acid treatment such as precipitate formation and the conflicting problems associated with the guanidine treatment, and thereby have completed the present  
30      invention. We have also found that it is further preferred to add a surfactant to the treatment agent comprising a chaotropic ion such as guanidine and an acidifying agent. As the acidifying agent, hydrochloric acid, sulfuric acid, acetic acid, trifluoroacetic acid, trichloroacetic acid, and the like are preferred.  
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As the surfactant, an amphoteric surfactant such as CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-

propanesulfonate), CHAPSO (3-[cholamidopropyl]dimethylammonio)-2-hydroxy-1-propanesulfonate), dodecyl-N-betaine, 3-(dodecyldimethylammonio)-1-propanesulfonate, or the like, and a nonionic surfactant such as a polyoxyethylene isoctylphenyl ether, for example Triton X100; a polyoxyethylene nonylphenyl ether, for example NP 40; a polyoxyethylene sorbitol ester, for example Tween 20; a polyoxyethylene dodecyl ether, for example Brij 58; octyl glucoside, or the like is preferred. Furthermore, an agent such as urea that partially destroys a higher structure of proteins by weakening hydrogen ion bonding may be added therein.

Especially, it is more preferred to use guanidine hydrochloride at 2 M or greater, Triton X100 at 2% or greater, and Tween 20 at 0.02% or greater at a temperature of 4 °C to 45 °C.

In any of the embodiments, it is evident that a virus antigen can be released in the form of a probe, i.e. a state suitable for the so-called immunoassay that uses antibody as a probe, from the sample containing virus particles having a structure similar to that of HCV or HBV by using the treating method of the present invention. Viruses having a structure similar to that of HCV or HBV as used herein are viruses that form virus particles having a structure composed of proteins in which the genomic RNA or DNA has been packed and the membrane protein or the lipid membrane surrounding it. The viruses include, for example, flaviviruses that are related to HCV, retroviruses such as human immunodeficiency virus (HIV), and the like. Furthermore, those having DNA as the genome like HBV are also included when they have a similar structure.

Exposure of virus antigen

According to the second embodiment of the present invention which relates to a method of detecting the virus antigen in a sample collected during the window period, antibody to the virus antigen has not been formed